ABSTRACT

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Title of Graduation Thesis: Optimalization and validation of SPE method for the determination of quercetin and its 9 metabolites in biological material.

The aim of this study was to develop and validate a new extraction method for the preparation of biological samples for the determination of quercetin and its 9 metabolites: phloroglucinol, 3,4-dihydroxyphenylacetic acid, homovanilic acid, 3-hydroxyphenylacetic acid, 3-(3-hydroxyphenyl)propionic acid, rutin, quercetin-3-glucuronide, tamarixetin and isorhamnetin.

Due to the best retention and subsequent elution of all tested analytes the mixed mode (polymeric ion exchanging) cartridge MAX was chosen. The mixture of methanol and 0.5 % trifluoracetic acid in water (95/5) was chosen as optimal solvent for elution. The combination of 0.01M ammonium formate buffer pH 5.0 and 1% methanol in buffer pH 5.0 were chosen as washing solvent.

The determination of quercetin and its 9 metabolites was performed using UHPLC-MS/MS. The best selectivity between the critical pair of analytes with the same molecular weight (tamarixetin and isorhamnetin) was achieved using BEH Shield RP18 column and gradient elution with methanol and 0.1% formic acid. The ionization was performed in electrospray polarity switching mode. The quantification was performed by triple quadrupole and selected reaction monitoring (SRM) mode. The method was validated in terms of linearity, sensitivity (LOD, LOQ), accuracy, precision, selectivity and matrix effects. Good linearity demonstrated correlation coefficients value ≥ 0.990. Method precision was expressed as RSD ≤ 20.0 %. The results of method accuracy were between 79.6 – 116.6 % except for three metabolites (PG, HVA, PAA) for which the results of accuracy 31.1 – 61.4 %. The advantage of newly developed SPE method for the preparation of biological samples prior to UHPLC-MS/MS analysis is simultaneous analysis and extraction of the compounds with different physicochemical properties.

Key words: UHPLC-MS/MS; SPE; quercetin; metabolites; method validation.